Normal Human Melanocyte Homeostasis as a Paradigm for Understanding Melanoma

Nikolas K. Haass and Meenhard Herlyn
The Wistar Institute, Philadelphia, Pennsylvania, USA

Melanocytes, after cell division, separate and migrate along the basement membrane; they extend their dendrites and establish multiple contacts with keratinocytes. Once adhesion is established, keratinocytes control melanocyte growth and expression of cell surface receptors. Most melanomas arise within the epidermis (melanoma *in situ*) and then invade across the basement membrane. These melanoma cells escape from control by keratinocytes through five major mechanisms: (1) downregulation of receptors important for communication with keratinocytes such as E-cadherin, P-cadherin, and desmoglein, which is achieved through growth factors such as hepatocyte growth factor, platelet-derived growth factor, and endothelin-1 produced by fibroblasts or keratinocytes; (2) upregulation of receptors and signaling molecules important for melanoma cell–melanoma cell and melanoma cell–fibroblast interactions such as N-cadherin, Mel-CAM, and zonula occludens protein-1; (3) deregulation of morphogens such as Notch receptors and their ligands; (4) loss of anchorage to the basement membrane because of an altered expression of cell–matrix adhesion molecules; (5) increased elaboration of metalloproteinases. Thus, investigating normal melanocyte homeostasis helps us to better define how melanoma cells escape the microenvironment created by epidermal keratinocytes and how they develop new cellular partners in fibroblasts and endothelial cells, which support their growth and invasion.

Key words: cell–cell communication/growth factors/metastasis/oncogenes/progression
J Investig Dermatol Symp Proc 10:153–163, 2005

In multicellular organisms, homeostasis is governed by either endocrine and paracrine communication via soluble factors including hormones, growth factors, and cytokines; or by intercellular communication via cell–cell and cell–matrix adhesion, and gap junctional intercellular communication (GJIC) (reviewed in Haass *et al*, 2004, 2005a). The characteristics of solid tumors, i.e., uncontrolled proliferation, derangement of cellular and morphological differentiation, invasion, and colonization to distant organs, can be attributed, in part, to alterations in intercellular communication between neoplastic cells and normal cells in their microenvironment (e.g., Hanahan and Weinberg, 2000; Park *et al*, 2000). Our laboratory is investigating melanocyte homeostasis in normal skin to gain a better understanding of deregulated growth patterns in melanomas because cancer is generally viewed as a result of the disruption of homeostatic regulations, which determine whether cells remain quiescent, proliferate, differentiate, or die.

The epidermal melanin unit of the human epidermis denotes the symbiotic relationship in which one melanocyte transports pigment-containing melanosomes through its dendrites to approximately 36 associated keratinocytes (Fitzpatrick and Breathnach, 1963; Jimbow *et al*, 1976). In this unit, melanocytes are located in the basal layer of the epidermis, where they keep a life-long stable ratio of 1:5 with basal keratinocytes (Fitzpatrick *et al*, 1979). This balance is maintained through regulated induction of melanocyte division and is only disturbed during transformation into a nevus or a melanoma. During childhood, i.e., during expansion of the total skin surface, there is a continuous need for melanocyte proliferation to maintain a stable ratio with the basal keratinocytes. In order to proliferate, melanocytes need to decouple from the basement membrane and from the keratinocytes, retract their dendrites, divide, and migrate along the basement membrane before they finally recouple to the matrix and to keratinocytes to form another epidermal melanin unit. The half-life of melanocytes is not well understood. But we assume that melanocytes during adulthood continue to proliferate albeit at a low rate and only upon specific stimulation such as sunlight exposure or wounding.

Physiologically, homeostasis determines whether a cell remains quiescent, proliferates, differentiates, or undergoes apoptosis (Bissell and Radisky, 2001). Dysregulation of the homeostasis may disturb the balance of the epidermal melanin unit and trigger a continuous proliferation of the melanocytes, which may lead to the development of melanoma. Hallmarks for melanoma cells include independence

Abbreviations: bFGF, basic fibroblast growth factor; ET-3, endothelin-3; GJIC, gap junctional intercellular communication; HGF, hepatocyte growth factor; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; RGP, radial growth phase; RTK, receptor tyrosine kinase; SCF, stem cell factor; TGF-β, transforming growth factor-β; UV, ultraviolet; VGP, vertical growth phase; ZO-1, zonula occludens protein-1

Copyright © 2005 by The Society for Investigative Dermatology, Inc.

from exogenous growth factors through the production of autocrine growth factors or the constitutive activation of growth factor-related signaling cascades, unlimited life span, escape from control by keratinocytes, unresponsiveness to inhibitory growth factors such as transforming growth factor-β (TGF-β), invasion into the dermis, recruitment of fibroblasts for matrix and growth factor production, and recruitment of a neovasculature (Hanahan and Weinberg, 2000). It is likely that melanoma cells escape from control by keratinocytes through the following mechanisms: (1) downregulation of receptors important for communication with keratinocytes, (2) upregulation of receptors and signaling molecules important for melanoma cell–melanoma cell and melanoma cell–fibroblast interactions, (3) deregulation of morphogens that directed them during development to the epidermis and helped maintain their proper position, (4) loss of anchorage to the basement membrane because of an altered expression of cell–matrix adhesion molecules, and (5) increased elaboration of metalloproteinases.

In this review we will focus on different steps during melanocyte proliferation within the complex microenvironment of the epidermis and how such studies help us to understand development of melanoma and its progression.

E-Cadherin-Mediated Regulation of Melanocytes

Under normal conditions in the epidermis, E-cadherin is expressed on the cell surface of both keratinocytes and melanocytes, and is the major adhesion molecule between epidermal melanocytes and keratinocytes (Tang *et al*, 1994; Hsu *et al*, 1996). Keratinocytes play an essential role in regulating melanocyte proliferation and differentiation (Hsu *et al*, 2000b). Normal melanocytes *in vitro* in monoculture have a cell surface phenotype similar to melanoma cells, i.e., they express most melanoma-associated antigens (Valyi-Nagy *et al*, 1993; Shih *et al*, 1994; Hsu *et al*, 2000b). When melanocytes are co-cultured with undifferentiated keratinocytes, expression of melanoma-associated antigens such as Mel-CAM and $\alpha_v\beta_3$ integrin is lost within 3–4 d, suggesting that the keratinocytes control cell surface molecules on the melanocytes (Shih *et al*, 1994). When keratinocytes and melanocytes are seeded together at a fixed ratio and allowed to proliferate, the original ratio remains constant during proliferation of both cell types, suggesting that the keratinocytes regulate their equilibrium with the melanocytes (Valyi-Nagy *et al*, 1993). These regulatory activities of keratinocytes occur through direct epidermal-specific cell–cell contact and not through soluble factors (Valyi-Nagy *et al*, 1993; Shih *et al*, 1994; Hsu *et al*, 2000b). E-cadherin is the most critical molecule that mediates keratinocyte–melanocyte interaction because melanocytes not expressing E-cadherin are not regulated by keratinocytes (Tang *et al*, 1994). Besides E-cadherin-mediated adherens junctions, desmosomes (*maculae adhaerentes*) are believed to play an important role in maintaining human skin homeostasis (Garrod, 1993). Reduced expression or switches of isoforms of desmosomal components seem to play a role in tumor progression (Tselepis *et al*, 1998; Chidgey, 2002). Although there are no desmosomes present in melanocytes, desmoglein 1 (Dsg1) is expressed in melanocytes and downregulated during melanoma genesis (Li *et al*, 2001b). Dsg1 has a similar expression pattern to E-cadherin and is a co-receptor for E-cadherin (Li *et al*, 2001b).

Most melanoma cells have lost expression of E-cadherin and instead have upregulated the expression of N-cadherin. This switch in cadherins is also known to change the binding partners of melanocytes from keratinocytes, so that they preferentially associate with fibroblasts and vascular endothelial cells (Hsu *et al*, 1996). These findings have been confirmed by clinical studies, which showed that both E-cadherin and P-cadherin are lost from vertical growth phase (VGP)-stage melanoma, with some samples also exhibiting increased N-cadherin expression (Sanders *et al*, 1999). On the other hand it has been shown *in vitro* and *in vivo* that E-cadherin is not always decreased in melanoma cells (Krengel *et al*, 2004; Tsutsumida *et al*, 2004). Our RT-PCR studies on 35 melanoma cell lines, revealed a number, which expressed both E- and N-cadherin at RNA level (Smalley *et al*, 2005). Most of the cell lines, which expressed E-cadherin at the RNA level, were radial growth phase (RGP) and early VGP melanomas. From these, a number of melanomas, which expressed E-cadherin at the RNA level, were tested for E-cadherin protein expression. Three out of the selected five melanoma cell lines, WM35, WM164, and WM1232 were found to express both E- and N-cadherin. But it was found in the WM35 and WM1232 cell lines that only N-cadherin was located at cell–cell adhesions. The expressed E-cadherin was largely restricted to cytoplasmic pools and thus not functional as an adhesion molecule (Smalley *et al*, 2005). Forced E-cadherin expression in melanoma cells, achieved by gene transfer, restores keratinocyte-mediated control, such as coordinated proliferation and downregulation of metastasis-related molecules Mel-CAM and $\alpha_v\beta_3$ integrin (Hsu *et al*, 2000b). E-cadherin-expressing melanoma cells, when introduced to skin reconstructs (skin equivalents, organotypic culture), remained in the epidermis and showed very little invasiveness into the dermis. The downregulation of E-cadherin and Dsg1 in melanocytes is also associated with gap junction incompatibility with keratinocytes (Hsu *et al*, 2000a). Forced expression of E-cadherin restores GJIC between melanoma cells and keratinocytes (Hsu *et al*, 2000a). Therefore, loss of E-cadherin appears to be one of the most critical steps in progression allowing melanoma cells to escape from the control through keratinocytes.

To dissect the possible mechanisms involved in the E-cadherin-mediated regulation of melanocytic cells by keratinocytes, a fusion protein between cytoplasmic domain-truncated E-cadherin and full-length α-catenin (Nagafuchi *et al*, 1994) was transduced into melanoma cells. This fusion gene prevents binding (and signaling) of β-catenin to E-cadherin while maintaining its adhesive functions and allows adhesion between melanoma cells and keratinocytes but it does not disrupt signaling for regulation of expression of melanoma-associated antigens, i.e., expression of Mel-CAM and β_3 integrin was downregulated in the same way as with wild-type E-cadherin (Li *et al*, 2004). On the other hand, if a dominant-negative mutant disrupts adhesion through E-cadherin, control no longer occurs (Li *et al*, 2004). These results suggest a predominant role for E-cadherin for adhesion. But it has not yet been ruled out whether

stabilization of β-catenin (and signaling for activation of TCF/LEF) occurs through the WNT pathway.

The transcriptional mechanisms for keratinocyte-mediated control over melanocytes and E-cadherin expressing melanoma cells are not yet clear. Cell–cell signaling is likely controlled by "master" genes that regulate expression of cell-surface molecules on the melanocytic cells and also control their growth. It has been shown that the Snail family of transcription factors represses E-cadherin expression and that Snail family proteins are expressed in melanoma and not melanocytes (Cano *et al*, 2000; Poser *et al*, 2001). The level of Snail expression correlates directly with the loss of E-cadherin expression. Forced expression of Snail in primary melanocytes downregulates E-cadherin expression (Poser *et al*, 2001). In carcinomas the transcription factor Twist, a master regulator for embryonic morphogenesis, is overexpressed and downregulates epithelial markers such as E-cadherin, α-catenin, and γ-catenin and upregulates mesenchymal markers such as N-cadherin, vimentin, and fibronectin; thus, Twist contributes to epithelial–mesenchymal transition (Kang and Massague, 2004; Yang *et al*, 2004). Furthermore, in melanoma an upregulation of Twist correlated with a poor prognosis (Hoek *et al*, 2004). Snail and Twist share the same target in the E-cadherin promoter (Nieto, 2002; Kang and Massague, 2004), suggesting that Twist represses E-cadherin in melanoma as well.

Knowledge of the activities of those "master" genes could help us understand how we may reestablish homeostasis in melanoma cells within distant metastases through "virtual keratinocytes," i.e., we can envision the development of small molecules that provide signals to the melanoma cells to down-modulate expression of invasion-related markers and to stop proliferation. Such therapeutic strategies may lead to stasis, not killing, similar to many related strategies that interfere with signaling.

Steps in the Melanocyte Proliferation Cascade

The unique position of melanocytes as singly aligned cells in the basal layer of the epidermis requires exquisite coordination for growth with keratinocytes. To better understand the likely cascade of events during proliferation, one can dissect them into four steps (Fig 1). The first two steps, decoupling from keratinocytes and cell division are described below in more detail because we know more about these. The third and fourth steps, migration to separate from each other and repositioning by extending dendrites into the upper layers of the epidermis, are still being investigated. Most likely integrins and morphogens such as Notch receptors and their ligands play determining roles for these processes, which are completed when the pigmented cells, upon stimulation by ultraviolet (UV) light, transport pigment to the surrounding keratinocytes. For this review, we will focus on the first two steps of the melanocyte proliferation cascade and the lessons we can learn for melanoma.

Decoupling of Melanocytes from Keratinocytes by Hepatocyte Growth Factor (HGF)-, Platelet-Derived Growth Factor (PDGF)-, and Endothelin-1 (ET-1)-Mediated Downregulation of E-Cadherin

In the skin, HGF (or scatter factor) is produced by fibroblasts for stimulation of keratinocytes and melanocytes. HGF/c-Met signaling has been implicated in the development of melanoma (Natali *et al*, 1993; Hendrix *et al*, 1998; Rusciano *et al*, 1998). HGF stimulates proliferation and motility of human melanocytes (Halaban *et al*, 1992; Kos *et al*, 1999). It is suggested that autocrine activation of the HGF receptor, c-Met, induces development of melanoma and acquisition of the metastatic phenotype (Otsuka *et al*, 1998). In melanocytes and melanoma cells, HGF decreases expression of both E-cadherin and desmoglein in a mitogen-activated protein kinase (MAPK) and PI3-kinase-dependent manner, leading to decoupling from keratinocytes (Li *et al*, 2001a). C-Met forms a complex with E-cadherin, desmoglein, and

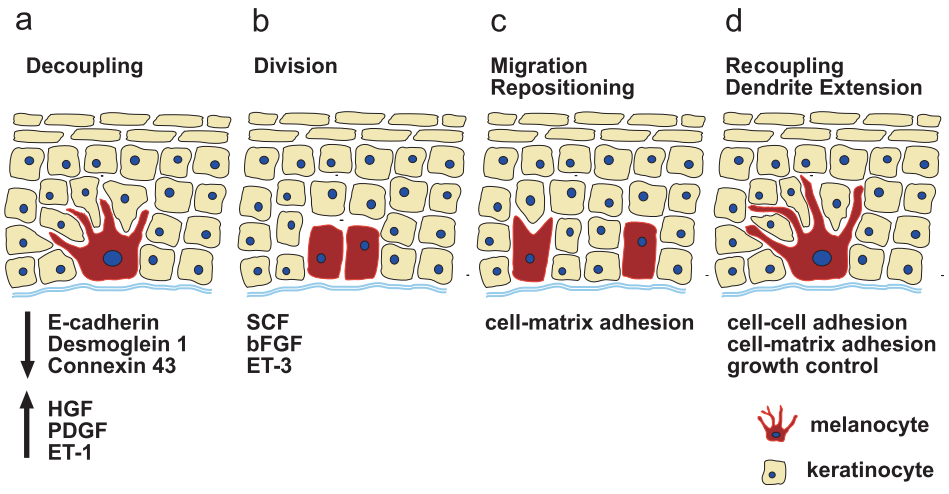

Figure 1
Melanocyte homeostasis in normal human skin. In order to proliferate, melanocytes need to decouple from the basement membrane and from the keratinocytes, retract their dendrites (*a*), divide (*b*), and migrate along the basement membrane (*c*) before they finally recouple to the matrix and to keratinocytes to form another epidermal melanin unit (*d*). Cell division for melanocytes likely begins with the upregulation of growth factors produced by either fibroblasts or keratinocytes, which lead to downregulation of cadherins and decoupling of melanocytes from keratinocytes. Mitogenesis is driven by other growth factors from dermis or epidermis. Migration for repositioning, anchorage to the basement membrane, recoupling to keratinocytes, dendrite extension into the upper cell layers, and growth control by keratinocytes complete the cycle for melanocytes to maintain a stable ratio with epidermal keratinocytes.

plakoglobin (Li *et al*, 2001a). The functional consequences of complex formation are not clear. Potentially, E-cadherin can down-modulate the biological functions of receptor tyrosine kinases (RTK) (Qian *et al*, 2004). Besides HGF, PDGF can also down-modulate E-cadherin in melanocytes (H. Schaider *et al*, unpublished), suggesting that the release of PDGF can also lead to decoupling of melanocytes from keratinocytes. Similarly, keratinocyte-mediated ET-1 can downregulate E-cadherin on melanocytes (Jamal and Schneider, 2002). These observations suggest the following: (1) E-cadherin is a very dynamic molecule that can relatively easily be down-modulated by microenvironmental factors, which also might explain why Langerhans cells can freely migrate through the epidermis despite expression of E-cadherin which likely allows them to establish communication with keratinocytes; and (2) changes in the skin microenvironment that locally enhance growth factor production may trigger decoupling of melanocytes from keratinocytes as a first step in deregulated (nevus) proliferation.

Proliferation of Melanocytes and Induction of Melanocytic Lesions in Human Skin Grafts with Growth Factors

We tested a variety of growth factors and cytokines for their ability to stimulate melanocytes in human skin grafted to immunodeficient SCID or RAG mice using adenoviral vectors for overexpression after intradermal injections (Berking *et al*, 2002; Gruss *et al*, 2003). The virus suspension spreads in the dermis and infects mostly fibroblasts (Berking *et al*, 2004). Strongest inducers of melanocyte proliferation were bFGF (basic fibroblast growth factors or FGF2) and SCF (stem cell factor or c-kit ligand). After a single injection, most of the 24 growth factors tested in the absence of UVB-induced changes in skin, including angiogenesis, matrix production, fibroblast growth stimulation, epidermal thickening, and/or inflammatory cell infiltration. These preliminary studies demonstrate that growth factors produced by dermal cells can stimulate melanocytes at the dermal/epidermal junction.

Overexpression of bFGF via adenoviral gene transfer in human skin xenografts led to black-pigmented macules within 3 wk of treatment which persisted for 8 mo under continuous weekly bFGF injections (Berking *et al*, 2001). Immunofluorescence analysis demonstrated pathological hyperpigmentation, proliferation, and hyperplasia of activated melanocytes but no malignant transformation (Berking *et al*, 2001). When bFGF was combined with UVB, pigmented lesions with hyperplastic melanocytic cells were detected, including a lesion with high-grade atypia resembling the lentiginous form of melanoma; this melanoma-like lesion appeared after approximately 10 wk (Berking *et al*, 2001). If UVB was replaced by UVA, we did not observe any UV-mediated effects on the melanocytes. When SCF was expressed in human skin grafted to SCID mice by intradermal injection, pigmented macules developed with increased numbers of melanocytes similar to the described effects of bFGF (Berking *et al*, 2004), whereas injection of ET-3/Ad5 did not induce striking melanocytic changes. UVB irradiation of the human skin together with the cutaneous expression of ET-3, SCF, or bFGF had no additional effect on the melanocytes within a 4 wk observation period. But a combination of bFGF, ET-3, and SCF led to cluster formation of the melanocytes in the epidermo-dermal junction zone and to migration of single melanocytes into the upper layers of the epidermis within 2–4 wk. After one injection of the three growth factors and three UVB irradiations at 30–50 mJ per cm^2, melanocytes started to proliferate and form nests in the basement membrane zone. When the weekly induced expression of all three growth factors bFGF, ET-3, and SCF was combined with 30–50 mJ per cm^2 UVB irradiation three times weekly, severe pigmented lesions developed (Berking *et al*, 2004) (Fig 2). Histologically these lesions were composed of atypical melanocytic cells and represented *in situ* melanomas or invasive melanomas resembling invasive and tumorigenic melanomas in patients' skin sections. Of the 79 human skin grafts that received the combination treatment, clinically pigmented lesions were observed in 89% and melanoma by histopathologic criteria in 34% (Table I).

Immunohistochemical analyses of the lesions revealed positivity for the melanoma markers S100, HMB45, Melan-A, and NKIC3. Experimental lesions showed histopathological similarities to those in patients. These data suggest that growth factors and UVB can induce melanoma-like lesions within a very short time. When adult skin from abdomen, breast or face was used instead of foreskin, we could also induce melanoma-like lesions. But these were milder, reflecting melanoma *in situ*. In contrast to foreskin, we could induce the lesions with growth factors only (Berking *et al*, 2004). Thus, in adult skin, UVB does not appear to have an additive effect, which indicates that juvenile skin is more susceptible to transforming effects by growth factors and UVB radiation. The correlation of UV and multiple compound nevi in humans might be explained by possibly preexisting acquired mutations in adult skin. Whether bFGF, SCF, and ET-3 are the only growth factors driving melanocyte proliferation synergistically, needs to be established. Potentially, other fibroblast or keratinocyte-derived growth factors or those with endocrine activities can drive proliferation and disturb the homeostatic balance.

bFGF has well-established functions in melanoma cells as a self-stimulatory growth factor, which can also be released by cells for stimulation of the vasculature and for stroma induction (Bikfalvi *et al*, 1997). The role of ET-1/ET-3 in melanoma is less clear. ET-1 is secreted by keratinocytes and stimulates proliferation, chemotaxis, and pigment production in melanocytic cells (Yada *et al*, 1991; Imokawa *et al*, 1992; Yohn *et al*, 1993, 1994; Imokawa *et al*, 1995, 1996; Horikawa *et al*, 1996). UV irradiation, which is strongly linked to melanoma development, induces a marked and sustained increase in ET-1 secretion by keratinocytes (Imokawa *et al*, 1992; Hara *et al*, 1995; Ahn *et al*, 1998). ET-3 is produced by bFGF-stimulated fibroblasts (Berking *et al*, 2004). Their receptor, ET_B receptor gradually increases expression as melanocytic lesions progress from nevi to melanomas, suggesting that the ET_B receptor, as well as its ligands play a role in the tumor progression of melanoma (Demunter *et al*, 2001). ET_B receptor blockade induces growth arrest and death of melanoma cells *in vivo* and *in vitro* (Lahav *et al*, 1999). ET_B receptor activation and caspase-8 activation through ET-1, secreted by keratinocytes

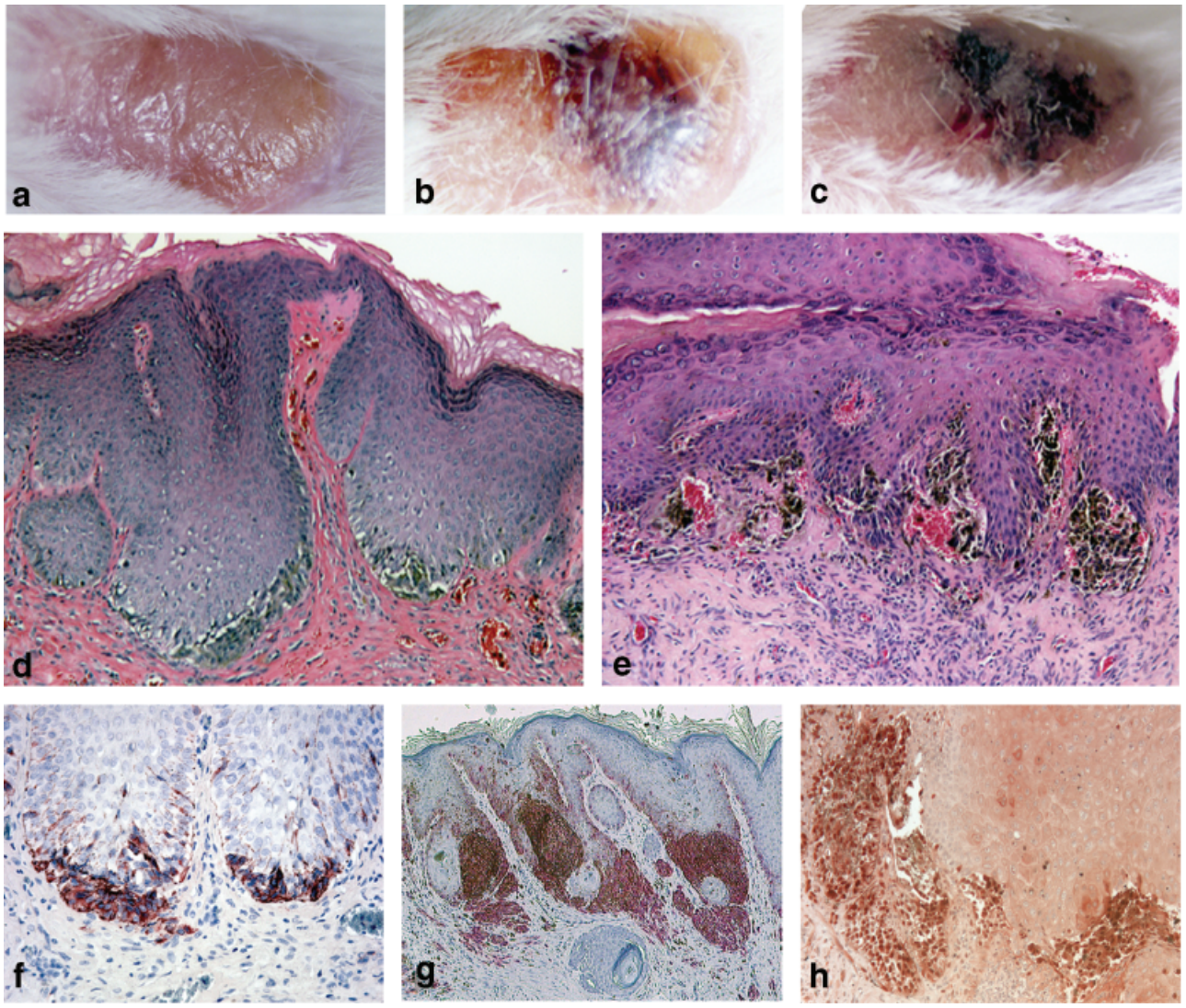

Figure 2
Melanoma-like lesions in human skin grafts induced by cutaneous expression of basic fibroblast growth factor, endothelin-3, and stem cell factor combined with irradiation with ultraviolet B. (*a*–*c*) Human skin graft on a SCID mouse before (*a*), 1 wk (*b*) and 3 wk after beginning of treatment (*c*). After 1 wk clinically, a pigmented lesion developed (*b*), which histologically showed hyperplasia of the epidermis and an increase in melanocytes, i.e. pigmented melanocytic nests (H&E staining in (*d*), HMB45 immuno-staining in (*f*)). After 4 wk melanoma-like lesions developed in human skin grafts (*c*), which were composed of big nests of transformed melanocytic cells that already broke through the basement membrane (H&E staining in (*e*)) and that stained positive for Melan A (*g*) and S100 (*h*).

in response to UV irradiation, downregulates E-cadherin in human melanocytes and melanoma cells (Jamal and Schneider, 2002). In melanoma cells ET_B receptor activation by ET-1 and ET-3 leads to a loss of E-cadherin, β-catenin, and p120, but to a gain of expression of the transcription factor Snail, N-cadherin, $\alpha_v\beta_3$, and $\alpha_2\beta_1$ integrin and, moreover, to the activation of the metalloproteinases MMP-2, MMP-9, and type-1-MMP and to the inhibition of GJIC because of phosphorylation of connexin 43 (Bagnato *et al*, 2004). Moreover, ET-1 upregulates MelCAM in melanocytes (Mangahas *et al*, 2004). All of these findings indicate that ET-1 mediates a variety of responses characteristic for dedifferentiation. At present, we cannot integrate in this model the observation that ET-1 induces dendricity in melanocytes (Hara *et al*, 1995), which is generally associated with differentiation. SCF does not appear to play a major role in melanoma, because most tumors show decreased expression of its receptor, c-kit (Lassam and Bickford, 1992; Natali *et al*, 1992; Zakut *et al*, 1993). Transduction of c-kit into melanoma cells and stimulation of cells with SCF may lead to cell death (Huang *et al*, 1996). In spontaneous melanoma it is possible that endogenous bFGF replaces exogenous SCF for stimulation of the appropriate signal transduction pathways.

Whether UVB induces genetic alterations in melanocytes is not clear yet but unlikely, because the exposure intensity was relatively mild and the short duration of the experiments would likely not induce genetic alteration in cells that promoted cell growth. Melanocytic cells isolated and cultured from five experimentally induced lesions formed colonies in soft agar comparable with cells from established melanoma lines while normal human melanocytes did not. But colony formation occurred only if the media were supplemented with the three growth factors, bFGF, ET-3, and SCF (Berking *et al*, 2004).

Melanocytic cells isolated and cultured from lesions showed increased resistance to the growth inhibitory

Table I. Pathologic changes in melanocytes in human skin grafts after 2–4 wk exposure to different growth factor combinations and UVB

Group[a]	Growth factors (n)	UVB	n	Pigmented lesion[b] (%)	Increase of melanocytes[c]	Suprabasal melanocytes[d]	Melanocytic nests[e]	Melanoma-like lesions[f] (%)
bFGF + ET-3 + SCF	3	+	79	70/79 (89)	4/5	21	5	17/50 (34)
		−	7	6/7	4/5	8	2	0
bFGF + ET-3	2	+	5	4/5	1/2	1	0	0
		−	5	3/5	2/5	6	0	0
bFGF + SCF	2	+	5	3/5	1/2	2	0	0
		−	5	3/5	2/5	4	1	0
ET-3 + SCF	2	+	5	0/5	1/4	0	0	0
		−	5	2/5	2/5	2	0	0
ET-3	1	+	5	0/5	1/5	0	0	0
		−	5	0/5	0/5	0	0	0
SCF	1	+	5	1/5	0/4	1	0	0
		−	5	3/5	2/4	3	0	0
bFGF	1	+	6	6/6	6/6	0	0	0
		−	6	3/6	3/6	0	0	0
GFP/LacZ	0	+	5	0/5	0/2	0	0	0
		−	5	0/5	1/4	0	0	0

[a]Growth factors bFGF, ET-3, and SCF as well as reporter genes GFP or LacZ were expressed in human skin grafts by weekly injections of the respective adenoviral vectors. The total dose of each injection was 5×10^8 PFU per graft in each group regardless of the number of growth factors used.
[b]Detection of at least one brown or black spot on the skin within 2–4 wk of treatment.
[c]Significant increase in HMB45-positive cells compared with non-treated skin grafts.
[d]Average number of melanocytic cells per histological section that have left the basement membrane and migrated into the epidermis.
[e]Average number of melanocytic nests per histological section. One nest is defined as a cluster of at least four melanocytic cells.
[f]Histopathological criteria are fulfilled to classify lesion as melanoma or melanoma *in situ*.
UV, ultraviolet; bFGF, basic fibroblast growth factors; ET-3, endothelin-3; SCF, stem cell factor.

effects of TGF-β1 when compared with normal melanocytes. But cultured cells had a finite life span. Similarly, the melanocytic lesions in the human skin grafts disappeared within 2–5 mo when growth factor injections and UVB irradiations were terminated.

These experiments established a model of human melanoma in a natural human skin environment that has resemblance to melanoma in patients. The lesions and the cells isolated and cultured from them fulfill most criteria for malignant transformation including: (1) insensitivity to growth inhibitory (anti-growth) signals, (2) evasion of programmed cell death (apoptosis), (3) sustained angiogenesis, (4) tissue invasion and metastasis. On the other hand, they continued to remain dependent on exogenous growth factors and had limited replicative potential, i.e., they senesced over time. Thus, our challenge is to induce tumorigenic (and metastatic) melanomas that fulfill all criteria of the "hallmarks of cancer".

The Genetic Base in Melanoma

The major cancer-related genes, including p53, $p16^{INK4A}$, or RAS, are rarely altered in melanoma although the pathways for these genes may not be biologically functional (reviewed in Satyamoorthy and Herlyn, 2002; Bogenrieder *et al*, 2003). Melanoma cells from advanced stage melanocytic lesions, including the VGP of primary melanoma are highly aneuploid, which has hampered a systematic analysis of the genetic basis of melanoma. The discovery in June of 2002 that the BRAF gene of the MAPK pathway is mutated in most sporadic melanomas (Brose *et al*, 2002; Davies *et al*, 2002) was a major breakthrough in the field. But BRAF is not only altered in melanoma but also in normal nevi (Pollock *et al*, 2003), suggesting that it is important for the switch from melanocytes to nevi but that additional genetic changes are needed to progress nevi to melanoma. In our melanoma studies it appears that growth factor overexpression can have synergistic biological effects with activating mutations in BRAF (Satyamoorthy *et al*, 2003). Thus, we can determine to what degree cells with BRAF mutations can become independent of exogenous growth factors. UVB likely induces additional, as yet unidentified changes at the biologic and genetic levels. But in contrast to melanoma in patients, cells in our model remain genetically relatively stable. Thus, it should be easier to determine specific genetic changes that promote malignant transformation.

Constitutive MAPK Activation in Melanoma

Activation of the MAPK pathway through growth factors that bind to RTK is a key component of the model of inducing melanoma-like lesions. Our previous studies with insulin-like growth factor-1 had implicated that the MAPK pathway

plays a critical role in melanoma survival and migration (Satyamoorthy *et al*, 2001a). We tested melanoma cell lines for Erk1/2 status under several experimental conditions. In normal melanocytes-activated Erk1/2 were not detectable under serum-free culture conditions. In contrast, all melanoma cell lines, regardless of their progression stages, clearly showed phosphorylated Erk1/2 even in the absence of serum. When fetal bovine serum was added to the culture medium, the phosphorylated form of Erk1/2 was induced in normal melanocytes, as well as in RGP and VGP cell lines. None of the metastatic melanoma lines responded to serum addition. The constitutive activation of Erk1/2 in melanoma remained under suspension culture condition. The results of ERK activation in melanoma were confirmed on lesions of patients. VGP primary and metastatic melanomas but not nevus lesions expressed phosphorylated ERK (Satyamoorthy *et al*, 2003).

The switch from growth factor dependence to independence is a major tumor progression step in melanoma. Nevi, which have frequent BRAF mutations (Pollock *et al*, 2003), still require exogenous growth factors and have a finite life span (Mancianti *et al*, 1988). The switch to growth factor independence distinguishes non-tumorigenic RGP from tumorigenic VGP primary melanomas and appears to be closely related to tumorigenicity and metastatic potential (Kath *et al*, 1991). Activation in melanoma of the MAPK pathway can be because of two mechanisms, autocrine production of the growth factors bFGF and HGF and through mutations in the BRAF gene, which occur in over 50% of melanomas (Brose *et al*, 2002; Davies *et al*, 2002). Despite the presence of BRAF mutations in melanoma cells, inhibition of expression or function of autocrine bFGF and HGF lead to downregulation of phosphorylation of ERK suggesting that activation of MAPK pathway members is because of synergistic activities of growth factors and genetic aberrations in selected genes such as $BRAF^{V600E}$.

The MAPK pathway is one of several pathways constitutively activated in melanoma cells. As illustrated (Fig 3), melanoma cells show activation, among others, also of the PI3 kinase pathways as well as of STAT3 and NF-κB signaling suggesting the existence of a number of endogenous or exogenous triggers. Their dissection will help us to develop novel strategies for therapy of melanoma. It is anticipated that successful therapy of melanoma directed at critical signaling pathways will encompass a combination of inhibitors.

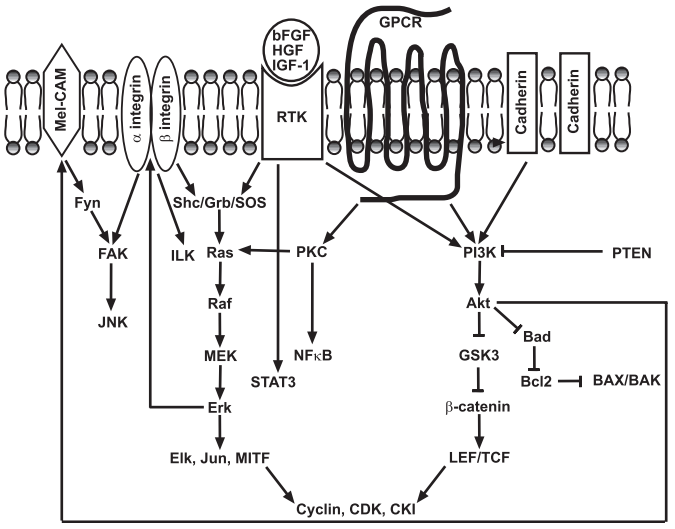

Figure 3
Intracellular signaling in melanoma. Binding of growth factors, matrix molecules, or other cells leads to activation of signaling cascades for several pathways including the MAPK PI3/AKT, STAT, or NF-κB pathways as potentially the most important networks with multiple interactions.

Cell–Cell Communication in Melanoma

Melanoma cells have lost E-cadherin expression but express high levels of N-cadherin *in vitro* and *in vivo* (Fig 4). The switching of cadherin subtypes during melanoma development enables melanoma cells to interact directly with other N-cadherin-expressing cells such as fibroblasts and vascular endothelial cells, thus affecting tumor-stroma cell adhesion, tumor cell invasion and migration, and gene expression. N-cadherin expression in melanoma cells allows communication with N-cadherin-expressing fibroblasts through gap junctions (Hsu *et al*, 2000a).

The downregulation of E-cadherin in melanocytes is associated with gap junction incompatibility with keratinocytes (Hsu *et al*, 2000a). Changes in the connexin expression, in particular the loss of Cx43, may result in a reduction or a loss of GJIC, which is thought to contribute towards tumor progression (reviewed in Haass *et al*, 2004). In the skin GJIC is likely to be involved in the regulation of keratinocyte growth, differentiation, and migration (Pitts *et al*, 1988; Salomon *et al*, 1993; Brissette *et al*, 1994; Goliger and Paul, 1995). We have shown in immunofluorescence studies on human tissue, that all areas of melanomas lack expression of the gap junction molecules connexin 26 (Cx26), Cx30, and Cx43. Interestingly, Cx26 and Cx30 are induced in the epidermis adjacent to melanomas, but are absent in the epidermis adjacent to melanocytic nevi (Haass *et al*, 2005b). Similarly, in other aggressive skin tumors—such as Merkel cell carcinoma and invasive areas of squamous cell, and Bowen's carcinoma—Cx26 and Cx30 are downregulated as well. There is also an induction of Cx26 and Cx30 in the epidermis adjacent to these tumors (Haass *et al*, 2003, 2005b).

N-cadherin also promotes migration of melanoma cells over dermal fibroblasts, suggesting that it plays a role in metastasis. In co-cultures of melanoma cells with fibroblasts, N-cadherin co-localizes with zonula occludens protein-1 (ZO-1) (Smalley *et al*, 2005). ZO-1 associates with claudins and occludins, and was first described as a component of tight junctions in simple epithelia, endothelia (Stevenson *et al*, 1986; Anderson *et al*, 1988) and, more recently, in stratified epithelia such as the epidermis (Pummi *et al*, 2001; Brandner *et al*, 2002, 2003; Furuse *et al*, 2002; Langbein *et al*, 2002). But ZO-1 has also been identified in non-epithelial cells, such as fibroblasts, where it instead associates with adherens junctions (Itoh *et al*, 1997; Giepmans and Moolenaar, 1998; Yokoyama *et al*, 2001). In these cadherin-based adherens junctions, ZO-1 functions as a cross-linker between α-catenin and the actin cytoskeleton (Itoh *et al*, 1997). ZO-1 forms protein–protein complexes with other molecules involved in cell–cell communication, such as the gap junction forming connexins (Kausalya *et al*,

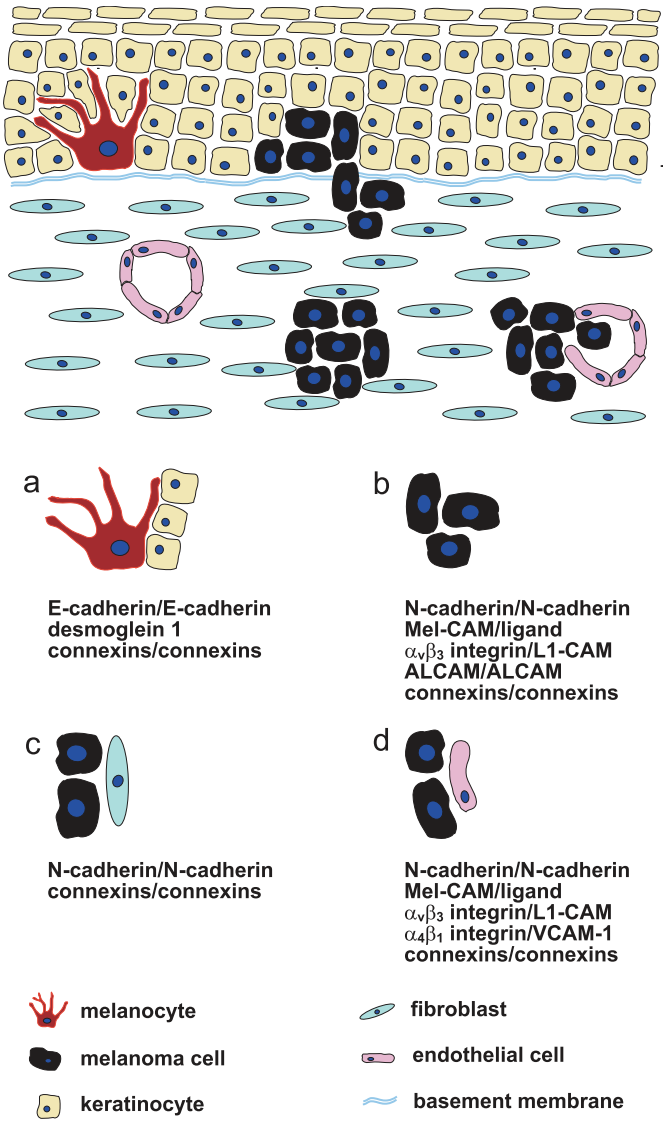

Figure 4
Cell–cell communication in melanoma. Normal epidermal melanocytes interact with adjacent keratinocytes through E-cadherin, desmoglein 1, and connexins (*a*). A shift of the cadherin profile from E- to N-cadherin during melanoma development not only frees the cells from epidermal keratinocytes, but also confers new adhesive and communication properties. Melanoma cells interact with each other (*b*) through N-cadherin, Mel-CAM/Mel-CAM ligand, $\alpha_v\beta_3$ integrin/L1-CAM, ALCAM (Degen *et al*, 1998; van Kempen *et al*, 2000) and connexins, with fibroblasts (*c*) through N-cadherin and connexins, and with endothelial cells (*d*) through N-cadherin, Mel-CAM/Mel-CAM ligand, $\alpha_v\beta_3$ integrin/L1-CAM, $\alpha_4\beta_1$ integrin/VCAM-1 (reviewed in Holzmann *et al*, 1998), and connexins.

2001; Laing *et al*, 2001; Nielsen *et al*, 2003). Usually ZO-1 is downregulated in malignant tumors (reviewed in Haass *et al*, 2004). Recently we demonstrated for the first time that expression ZO-1 is upregulated in Merkel cell carcinoma (Haass *et al*, 2003b) and in both melanoma cell lines and melanoma samples, and functionally associates with both N-cadherin and the actin cytoskeleton (Smalley *et al*, 2005). RNAi studies reveal that the strength of cell–cell adhesion is reduced when ZO-1 is knockeddown. In addition, ZO-1 loss is associated with altered cytoskeletal organization, lack of long pseudopodia and reduced invasion into collagen gels. It therefore seems that following cadherin switching, recruitment of increased levels of ZO-1 to N-cadherin-based junctions may reorganize the cytoskeleton, leading to greater invasion (Smallen *et al*, 2005).

In melanoma cell–melanoma cell interactions, the cell–cell adhesion molecule Mel-CAM and its unidentified ligand appear to play a role as co-receptor for N-cadherin, because its down-modulation disrupts GJIC (Satyamoorthy *et al*, 2001b). But Mel-CAM does not have major adhesive function but is instead involved in invasion and metastasis.

The Melanoma Progression Model—An Expanding View

Traditionally, the development of melanoma and its progression is described in six steps: (1) common acquired melanocytic nevus; (2) melanocytic nevus with lentiginous melanocytic hyperplasia, i.e., aberrant differentiation; (3) melanocytic nevus with aberrant differentiation, and melanocytic nuclear atypia, i.e., melanocytic dysplasia; (4) the RGP of primary melanoma; (5) the VGP of primary melanoma; and (6) metastatic melanoma (Clark *et al*, 1984). Our laboratory has defined cells from each progression step and has established biologic criteria for each step using unique *in vitro* and *in vivo* models. The stepwise progression model is widely accepted by the scientific community and many researchers have contributed to knowledge on the genetic, biologic, and molecular events that drive progression. Major gaps in knowledge still exist in tumor- and host-derived factors that progress melanoma cells from the RGP to the VGP step, a progression step that is most critical for clinical outcome of the disease. Most melanomas arise within the epidermis (melanoma *in situ*) and then invade across the basement membrane region. There may in addition be rare melanomas that arise *de novo* in the dermis in a subset of nodular melanomas and other at least equally rare melanomas that arise in the dermis in association with a pre-existing congenital nevus. Many melanomas (perhaps 30%–50%) arise in association with a putative precursor lesion as judged histologically. On the other hand, tumors arising without clinical precursor lesions represent approximately 50% of all sporadic melanoma cases and we have a limited understanding of the events that occur when melanomas arise without clinically defined precursor stages (Fig 5). We are currently exploring whether melanomas can arise from melanocyte precursor cells hypothesizing that such cells may transform without passing through the "classical" progression steps. The immature phenotype of melanoma cells may not necessarily come from dedifferentiation (this view) but also from transformation of melanocyte stem cells or precursor cells.

Fibroblasts within the tumor stroma may play an important role for progression of melanomas from RGP to VGP or from VGP to metastasis. We are currently testing the hypothesis that fibroblasts and their cross-talk to tumor cells are critical for disease outcome. We expect that fibroblasts infiltrating into tumors can be targeted for melanoma therapy because our preliminary investigations have demonstrated that their presence is essential for tumor

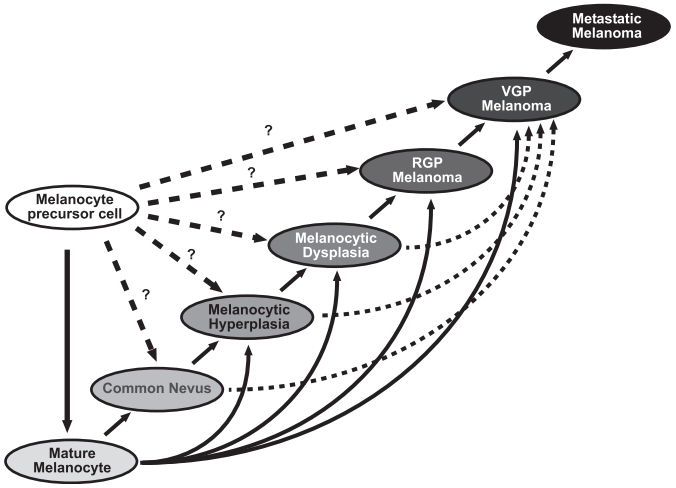

Figure 5
The six steps of the development of melanoma and its progression. (1) Common acquired melanocytic nevus; (2) melanocytic nevus with lentiginous melanocytic hyperplasia; (3) melanocytic nevus with melanocytic dysplasia; (4) the radial growth phase of primary melanoma; (5) the vertical growth phase of primary melanoma; and (6) metastatic melanoma (Clark *et al*, 1984). The fact, that in 50% of all sporadic melanoma cases tumors arise without clinical precursor lesions, leads to the hypothesis that those lesions might derive directly from mature melanocytes (*solid arrows*) or melanocyte precursor cells (*dashed arrows*). Most melanomas arise within the epidermis (melanoma *in situ*) and then invade across the basement membrane region. There may in addition be rare melanomas that arise *de novo*—presumably from melanocyte precursor cells—in the dermis in a subset of nodular melanomas and other at least equally rare melanomas that arise in the dermis in association with a pre-existing congenital nevus (shown as dotted arrows from "Common Nevus/Melanocytic Hyperplasia/Melanocytic Dysplasia" directly to "VGP Melanoma").

expansion beyond an initial cell cluster. We also expect that fibroblasts in the dermis have a major role in melanocyte stem cell differentiation and that their dysregulation initiates tumor formation. Furthermore, we expect that stromal fibroblasts play a role in melanoma stem cell maintenance, most likely through the production of growth factors. Identification of melanoma stem cells should lead to new strategies for induction of differentiation of the most aggressive subpopulations within a tumor.

We are developing an expanding view on the role of endothelial cells in melanoma progression and metastasis. The cell type traditionally thought to be involved in the tumor vascularization is the microvascular endothelial cell, previously believed to derive primarily from resident tissue such as skin. But circulating endothelial precursor cells were isolated from adult blood and bone marrow and once they become adherent *in vitro* these cells differentiated into mature endothelial cells (Asahara *et al*, 1997). The precursor cells were found in the endothelial cell population of developing tumors, healing wounds, ischemic myocardium, and ischemic hind limbs (Asahara *et al*, 1997, 1999; Takahashi *et al*, 1999). Locally transplanted autologous bone marrow-derived mononuclear cells that contain endothelial precursor cells survived and were successfully incorporated into the capillary endothelial networks during active angiogenesis *in vivo* and augmented neo-vascularization and collateral vessel formation (Kamihata *et al*, 2001). Most recent data define a dendritic precursor cell that can develop into endothelial cells as it infiltrates tumors (Conejo-Garcia *et al*, 2004, 2005). The endothelial-like cells may have also retain some of their original immunological functions such as antigen presentation suggested that the tumors represent an "organ" with complex intercellular communications and that tumor progression may in large parts depend on appropriate cooperation or submission of the normal cell components.

We thank Dr Sumayah Jamal (New York) and Dr David Elder (Philadelphia) for suggestions and all members of Dr Meenhard Herlyn's lab for intellectual support. We apologize to all those colleagues whose important work we could not cite because of space limitations.

DOI: 10.1111/j.1087-0024.2005.200407.x

Manuscript received January 12, 2005; accepted for publication June 2, 2005

Address correspondence to: Dr Meenhard Herlyn, The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104, USA. Email: herlynm@wistar.org

References

Ahn GY, Butt KI, Jindo T, Yaguchi H, Tsuboi R, Ogawa H: The expression of endothelin-1 and its binding sites in mouse skin increased after ultraviolet B irradiation or local injection of tumor necrosis factor alpha. J Dermatol 25:78–84, 1998

Anderson JM, Stevenson BR, Jesaitis LA, Goodenough DA, Mooseker MS: Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin–Darby canine kidney cells. J Cell Biol 106:1141–1149, 1988

Asahara T, Murohara T, Sullivan A, *et al*: Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967, 1997

Asahara T, Takahashi T, Masuda H, *et al*: VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 18:3964–3972, 1999

Bagnato A, Rosano L, Spinella F, Di Castro V, Tecce R, Natali PG: Endothelin B receptor blockade inhibits dynamics of cell interactions and communications in melanoma cell progression. Cancer Res 64:1436–1443, 2004

Berking C, Takemoto R, Binder RL, *et al*: Photocarcinogenesis in human adult skin grafts. Carcinogenesis 23:181–187, 2002

Berking C, Takemoto R, Satyamoorthy K, *et al*: Induction of melanoma phenotypes in human skin by growth factors and ultraviolet B. Cancer Res 64:807–811, 2004

Berking C, Takemoto R, Satyamoorthy K, Elenitsas R, Herlyn M: Basic fibroblast growth factor and ultraviolet B transform melanocytes in human skin. Am J Pathol 158:943–953, 2001

Bikfalvi A, Klein S, Pintucci G, Rifkin DB: Biological roles of fibroblast growth factor-2. Endocr Rev 18:26–45, 1997

Bissell MJ, Radisky D: Putting tumours in context. Nat Rev Cancer 1:46–54, 2001

Bogenrieder T, Elder D, Herlyn M: Molecular and cellular biology. In: Balch CM, Houghton AN, Sober AJ, Soong SJ (eds). Cutaneous Melanoma. St Louis: Quality Medical Publishing Inc., 2003; p 713–751

Brandner JM, Kief S, Grund C, *et al*: Organization and formation of the tight junction system in human epidermis and cultured keratinocytes. Eur J Cell Biol 81:253–263, 2002

Brandner JM, McIntyre M, Kief S, Wladykowski E, Moll I: Expression and localization of tight junction-associated proteins in human hair follicles. Arch Dermatol Res 295:211–221, 2003

Brissette JL, Kumar NM, Gilula NB, Hall JE, Dotto GP: Switch in gap junction protein expression is associated with selective changes in junctional permeability during keratinocyte differentiation. Proc Natl Acad Sci USA 91:6453–6457, 1994

Brose MS, Volpe P, Feldman M, *et al*: BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 62:6997–7000, 2002

Cano A, Perez-Moreno MA, Rodrigo I, *et al*: The transcription factor snail controls epithelial–mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2:76–83, 2000

Chidgey M: Desmosomes and disease: An update. Histol Histopathol 17: 1179–1192, 2002

Clark WH Jr, Elder DE, Guerry DT, Epstein MN, Greene MH, Van Horn M: A study of tumor progression: The precursor lesions of superficial spreading and nodular melanoma. Hum Pathol 15:1147–1165, 1984

Conejo-Garcia JR, Benencia F, Courreges MC, *et al*: Tumor-infiltrating dendritic cell precursors recruited by a beta-defensin contribute to vasculogenesis under the influence of Vegf-A. Nat Med 10:950–958, 2004

Conejo-Garcia JR, Buckanovich RJ, Benencia F, Courreges MC, Rubin SC, Carroll RG, Coukos G: Vascular leukocytes contribute to tumor vascularization. Blood 105:679–681, 2005

Davies H, Bignell GR, Cox C, *et al*: Mutations of the BRAF gene in human cancer. Nature 417:949–954, 2002

Degen WG, van Kempen LC, Gijzen EG, van Groningen JJ, van Kooyk Y, Bloemers HP, Swart GW: MEMD, a new cell adhesion molecule in metastasizing human melanoma cell lines, is identical to ALCAM (activated leukocyte cell adhesion molecule). Am J Pathol 152:805–813, 1998

Demunter A, De Wolf-Peeters C, Degreef H, Stas M, van den Oord JJ: Expression of the endothelin-B receptor in pigment cell lesions of the skin. Evidence for its role as tumor progression marker in malignant melanoma. Virchows Arch 438:485–491, 2001

Fitzpatrick TB, Breathnach AS: The epidermal melanin unit system. Dermatol Wochenschr 147:481–489, 1963

Fitzpatrick TB, Szabo G, Seiji M, Quevedo WC Jr: Biology of the melanin pigmentary system. In: Fitzpatrick TB, Eisen A, Wolff K, Freedberg I, Austen K (eds). Dermatology in General Medicine. New York: McGraw-Hill, 1979; p 131–145

Furuse M, Hata M, Furuse K, *et al*: Claudin-based tight junctions are crucial for the mammalian epidermal barrier: A lesson from claudin-1-deficient mice. J Cell Biol 156:1099–1111, 2002

Garrod DR: Desmosomes and hemidesmosomes. Curr Opin Cell Biol 5:30–40, 1993

Giepmans BN, Moolenaar WH: The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. Curr Biol 8:931–934, 1998

Goliger JA, Paul DL: Wounding alters epidermal connexin expression and gap junction-mediated intercellular communication. Mol Biol Cell 6:1491–1501, 1995

Gruss CJ, Satyamoorthy K, Berking C, *et al*: Stroma formation and angiogenesis by overexpression of growth factors, cytokines, and proteolytic enzymes in human skin grafted to SCID mice. J Invest Dermatol 120:683–692, 2003

Haass NK, Houdek P, Brandner JM, Moll I: Expression patterns of connexins in Merkel cell carcinoma and adjacent epidermis. In: Baumann KI, Moll I, Halata Z (eds). The Merkel Cell—Structure–Development–Function—and Cancerogenesis. Berlin: Springer-Verlag, 2003a; p 219–222

Haass NK, Houdek P, Wladykowski E, Moll I, Brandner JM: Expression patterns of tight junction proteins in Merkel cell carcinoma. In: Baumann KI, Moll I, Halata Z (eds). The Merkel Cell—Structure–Development–Function—and Cancerogenesis. Berlin: Springer-Verlag, 2003b; p 223–226

Haass NK, Smalley KSM, Herlyn M: The role of altered cell–cell communication in melanoma progression. J Mol Histol 35:309–318, 2004

Haass NK, Smalley KSM, L L, Herlyn M: Adhesion, migration and communication in melanocytes and melanoma. Pigment Cell Res 18:150–159, 2005a

Haass NK, Wladykowski E, Kief S, Moll I, Brandner JM: Differential induction of connexins 26 and 30 in skin tumors and their adjacent epidermis. J Histochem Cytochem doi:10.1369/jhc.5A6719.2005, 2005b

Halaban R, Rubin JS, Funasaka Y, *et al*: Met and hepatocyte growth factor/scatter factor signal transduction in normal melanocytes and melanoma cells. Oncogene 7:2195–2206, 1992

Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 100:57–70, 2000

Hara M, Yaar M, Gilchrest BA: Endothelin-1 of keratinocyte origin is a mediator of melanocyte dendricity. J Invest Dermatol 105:744–748, 1995

Hendrix MJ, Seftor EA, Seftor RE, *et al*: Regulation of uveal melanoma interconverted phenotype by hepatocyte growth factor/scatter factor (HGF/SF). Am J Pathol 152:855–863, 1998

Hoek K, Rimm DL, Williams KR, *et al*: Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. Cancer Res 64:5270–5282, 2004

Holzmann B, Gosslar U, Bittner M: Alpha 4 integrins and tumor metastasis. Curr Top Microbiol Immunol 231:125–141, 1998

Horikawa T, Norris DA, Zekman T, Morelli JG: Effective elimination of fibroblasts in cultures of melanocytes by lowering calcium concentration in TPA depleted medium following geneticin treatment. Pigment Cell Res 9:58–62, 1996

Hsu M, Andl T, Li G, Meinkoth JL, Herlyn M: Cadherin repertoire determines partner-specific gap junctional communication during melanoma progression. J Cell Sci 113 (Part 9):1535–1542, 2000a

Hsu MY, Meier FE, Nesbit M, Hsu JY, Van Belle P, Elder DE, Herlyn M: E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors. Am J Pathol 156:1515–1525, 2000b

Hsu MY, Wheelock MJ, Johnson KR, Herlyn M: Shifts in cadherin profiles between human normal melanocytes and melanomas. J Investig Dermatol Symp Proc 1:188–194, 1996

Huang S, Luca M, Gutman M, McConkey DJ, Langley KE, Lyman SD, Bar-Eli M: Enforced c-KIT expression renders highly metastatic human melanoma cells susceptible to stem cell factor-induced apoptosis and inhibits their tumorigenic and metastatic potential. Oncogene 13:2339–2347, 1996

Imokawa G, Miyagishi M, Yada Y: Endothelin-1 as a new melanogen: Coordinated expression of its gene and the tyrosinase gene in UVB-exposed human epidermis. J Invest Dermatol 105:32–37, 1995

Imokawa G, Yada Y, Kimura M: Signalling mechanisms of endothelin-induced mitogenesis and melanogenesis in human melanocytes. Biochem J 314 (Part 1):305–312, 1996

Imokawa G, Yada Y, Miyagishi M: Endothelins secreted from human keratinocytes are intrinsic mitogens for human melanocytes. J Biol Chem 267:24675–24680, 1992

Itoh M, Nagafuchi A, Moroi S, Tsukita S: Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. J Cell Biol 138:181–192, 1997

Jamal S, Schneider RJ: UV-induction of keratinocyte endothelin-1 downregulates E-cadherin in melanocytes and melanoma cells. J Clin Invest 110:443–452, 2002

Jimbow K, Quevedo WC Jr, Fitzpatrick TB, Szabo G: Some aspects of melanin biology: 1950–1975. J Invest Dermatol 67:72–89, 1976

Kamihata H, Matsubara H, Nishiue T, *et al*: Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation 104:1046–1052, 2001

Kang Y, Massague J: Epithelial–mesenchymal transitions: Twist in development and metastasis. Cell 118:277–279, 2004

Kath R, Jambrosic JA, Holland L, Rodeck U, Herlyn M: Development of invasive and growth factor-independent cell variants from primary human melanomas. Cancer Res 51:2205–2211, 1991

Kausalya PJ, Reichert M, Hunziker W: Connexin45 directly binds to ZO-1 and localizes to the tight junction region in epithelial MDCK cells. FEBS Lett 505:92–96, 2001

Kos L, Aronzon A, Takayama H, Maina F, Ponzetto C, Merlino G, Pavan W: Hepatocyte growth factor/scatter factor-MET signaling in neural crest-derived melanocyte development. Pigment Cell Res 12:13–21, 1999

Krengel S, Groteluschen F, Bartsch S, Tronnier M: Cadherin expression pattern in melanocytic tumors more likely depends on the melanocyte environment than on tumor cell progression. J Cutan Pathol 31:1–7, 2004

Lahav R, Heffner G, Patterson PH: An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells *in vitro* and *in vivo*. Proc Natl Acad Sci USA 96:11496–11500, 1999

Laing JG, Manley-Markowski RN, Koval M, Civitelli R, Steinberg TH: Connexin45 interacts with zonula occludens-1 and connexin43 in osteoblastic cells. J Biol Chem 276:23051–23055, 2001

Langbein L, Grund C, Kuhn C, *et al*: Tight junctions and compositionally related junctional structures in mammalian stratified epithelia and cell cultures derived therefrom. Eur J Cell Biol 81:419–435, 2002

Lassam N, Bickford S: Loss of c-kit expression in cultured melanoma cells. Oncogene 7:51–56, 1992

Li G, Fukunaga M, Herlyn M: Reversal of melanocytic malignancy by keratinocytes is an E-cadherin-mediated process overriding beta-catenin signaling. Exp Cell Res 297:142–151, 2004

Li G, Satyamoorthy K, Herlyn M: N-cadherin-mediated intercellular interactions promote survival and migration of melanoma cells. Cancer Res 61:3819–3825, 2001a

Li G, Schaider H, Satyamoorthy K, Hanakawa Y, Hashimoto K, Herlyn M: Down-regulation of E-cadherin and Desmoglein 1 by autocrine hepatocyte growth factor during melanoma development. Oncogene 20:8125–8135, 2001b

Mancianti ML, Herlyn M, Weil D, *et al*: Growth and phenotypic characteristics of human nevus cells in culture. J Invest Dermatol 90:134–141, 1988

Mangahas CR, dela Cruz GV, Schneider RJ, Jamal S: Endothelin-1 upregulates MCAM in melanocytes. J Invest Dermatol 123:1135–1139, 2004

Nagafuchi A, Ishihara S, Tsukita S: The roles of catenins in the cadherin-mediated cell adhesion: Functional analysis of E-cadherin-alpha catenin fusion molecules. J Cell Biol 127:235–245, 1994

Natali PG, Nicotra MR, Di Renzo MF, Prat M, Bigotti A, Cavaliere R, Comoglio PM: Expression of the c-Met/HGF receptor in human melanocytic neoplasms: Demonstration of the relationship to malignant melanoma tumour progression. Br J Cancer 68:746–750, 1993

Natali PG, Nicotra MR, Winkler AB, Cavaliere R, Bigotti A, Ullrich A: Progression of human cutaneous melanoma is associated with loss of expression of c-kit proto-oncogene receptor. Int J Cancer 52:197–201, 1992

Nielsen PA, Baruch A, Shestopalov VI, Giepmans BN, Dunia I, Benedetti EL, Kumar NM: Lens connexins alpha3Cx46 and alpha8Cx50 interact with zonula occludens protein-1 (ZO-1). Mol Biol Cell 14:2470–2481, 2003

Nieto MA: The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 3:155–166, 2002

Otsuka T, Takayama H, Sharp R, *et al*: c-Met autocrine activation induces development of malignant melanoma and acquisition of the metastatic phenotype. Cancer Res 58:5157–5167, 1998

Park CC, Bissell MJ, Barcellos-Hoff MH: The influence of the microenvironment on the malignant phenotype. Mol Med Today 6:324–329, 2000

Pitts JD, Finbow ME, Kam E: Junctional communication and cellular differentiation. Br J Cancer 9 (Suppl.):52–57, 1988

Pollock PM, Harper UL, Hansen KS, *et al*: High frequency of BRAF mutations in nevi. Nat Genet 33:19–20, 2003

Poser I, Dominguez D, de Herreros AG, Varnai A, Buettner R, Bosserhoff AK: Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. J Biol Chem 276:24661–24666, 2001

Pummi K, Malminen M, Aho H, Karvonen SL, Peltonen J, Peltonen S: Epidermal tight junctions: ZO-1 and occludin are expressed in mature, developing, and affected skin and *in vitro* differentiating keratinocytes. J Invest Dermatol 117:1050–1058, 2001

Qian X, Karpova T, Sheppard AM, McNally J, Lowy DR: E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases. EMBO J 23:1739–1784, 2004

Rusciano D, Lorenzoni P, Lin S, Burger MM: Hepatocyte growth factor/scatter factor and hepatocytes are potent downregulators of tyrosinase expression in B16 melanoma cells. J Cell Biochem 71:264–276, 1998

Salomon D, Masgrau E, Vischer S, Chanson M, Saurat JH, Spray DS, Meda P: Gap junction proteins and communication in human epidermis. Prog Cell Res 3:255–231, 1993

Sanders DS, Blessing K, Hassan GA, Bruton R, Marsden JR, Jankowski J: Alterations in cadherin and catenin expression during the biological progression of melanocytic tumours. Mol Pathol 52:151–157, 1999

Satyamoorthy K, Herlyn M: Cellular and molecular biology of human melanoma. Cancer Biol Ther 1:14–17, 2002

Satyamoorthy K, Li G, Gerrero MR, *et al*: Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. Cancer Res 63:756–759, 2003

Satyamoorthy K, Li G, Vaidya B, Patel D, Herlyn M: Insulin-like growth factor-1 induces survival and growth of biologically early melanoma cells through both the mitogen-activated protein kinase and beta-catenin pathways. Cancer Res 61:7318–7324, 2001a

Satyamoorthy K, Muyrers J, Meier F, Patel D, Herlyn M: Mel-CAM-specific genetic suppressor elements inhibit melanoma growth and invasion through loss of gap junctional communication. Oncogene 20:4676–4684, 2001b

Shih IM, Elder DE, Hsu MY, Herlyn M: Regulation of Mel-CAM/MUC18 expression on melanocytes of different stages of tumor progression by normal keratinocytes. Am J Pathol 145:837–845, 1994

Smalley KS, Brafford P, Haass NK, Brandner JM, Brown E, Herlyn M: Up-regulated expression of zonula occludens protein-1 in human melanoma associates with N-cadherin and contributes to invasion and adhesion. Am J Pathol 166:1541–1554, 2005

Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA: Identification of ZO-1: A high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol 103:755–766, 1986

Takahashi T, Kalka C, Masuda H, *et al*: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 5:434–438, 1999

Tang A, Eller MS, Hara M, Yaar M, Hirohashi S, Gilchrest BA: E-cadherin is the major mediator of human melanocyte adhesion to keratinocytes *in vitro*. J Cell Sci 107 (Part 4):983–992, 1994

Tselepis C, Chidgey M, North A, Garrod D: Desmosomal adhesion inhibits invasive behavior. Proc Natl Acad Sci USA 95:8064–8069, 1998

Tsutsumida A, Hamada J, Tada M, *et al*: Epigenetic silencing of E- and P-cadherin gene expression in human melanoma cell lines. Int J Oncol 25:1415–1421, 2004

Valyi-Nagy IT, Hirka G, Jensen PJ, Shih IM, Juhasz I, Herlyn M: Undifferentiated keratinocytes control growth, morphology, and antigen expression of normal melanocytes through cell–cell contact. Lab Invest 69:152–159, 1993

van Kempen LC, van den Oord JJ, van Muijen GN, Weidle UH, Bloemers HP, Swart GW: Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin. Am J Pathol 156:769–774, 2000

Yada Y, Higuchi K, Imokawa G: Effects of endothelins on signal transduction and proliferation in human melanocytes. J Biol Chem 266:18352–18357, 1991

Yang J, Mani SA, Donaher JL, *et al*: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117:927–939, 2004

Yohn JJ, Morelli JG, Walchak SJ, Rundell KB, Norris DA, Zamora MR: Cultured human keratinocytes synthesize and secrete endothelin-1. J Invest Dermatol 100:23–26, 1993

Yohn JJ, Smith C, Stevens T, *et al*: Human melanoma cells express functional endothelin-1 receptors. Biochem Biophys Res Commun 201:449–457, 1994

Yokoyama S, Tachibana K, Nakanishi H, *et al*: alpha-catenin-independent recruitment of ZO-1 to nectin-based cell–cell adhesion sites through afadin. Mol Biol Cell 12:1595–1609, 2001

Zakut R, Perlis R, Eliyahu S, Yarden Y, Givol D, Lyman SD, Halaban R: KIT ligand (mast cell growth factor) inhibits the growth of KIT-expressing melanoma cells. Oncogene 8:2221–2229, 1993